Effect of an Aphelenchoides Species on the Growth of a Mycorrhizal and a Pseudomycorrhizal Fungus

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ABSTRACT

An undescribed *Aphelenchoides* species greatly reduced the diameter growth of the aerial and substrate mycelium of *Suillus granulatus* and *Mycelium radicis atrovirens* in two laboratory experiments. The nematode reproduced readily on both fungi and destroyed 87% of the *S. granulatus* cultures; *M. radicis atrovirens* cultures, however, were not destroyed. Nematodes were not able to maintain themselves in cultures that contained an agar medium without the fungi.

Several nematode species are known to parasitize the mycorrhizae of conifers. *Criconemoides rusticum* (Micoletzky, 1915) Taylor, 1936 was associated with the deterioration of fine roots and mycorrhizae of *Pinus echinata* Mill. seedlings (4). *Hoplolaimus tylenchiformis* Daday, 1905 and *Meloidodera floridensis* Chitwood et al., 1956 have parasitized lateral roots and mycorrhizae of *Pinus taeda* L. and *P. elliottii* Engelm. (5).

Although nematodes parasitize mycorrhizae, there are few reports of nematodes feeding directly on mycorrhizal fungi. Clark (2) has presented circumstantial evidence that a *Deladenus* species may be responsible for the non-establishment of *Rhododendron* cuttings with mycorrhizae on their roots. He suggested that the nematode fed directly on the mycorrhizal fungus and destroyed the external mycelium as rapidly as it developed.

Myceliophagous nematodes have often been isolated from mycorrhizal rootlets of southwestern tree species. The study reported here was made to determine the effects of one of these nematodes on the growth of *Suillus granulatus* (Fries) Kuntze, a mycorrhizal fungus, and *Mycelium radicis atrovirens* Melin, a pseudomycorrhizal fungus.

MATERIALS AND METHODS.—The nematode and fungi used in the investigation were obtained from low-elevation stands of *Pinus ponderosa* Laws., *P. edulis* Engelm., and *Juniperus monosperma* (Engelm.) Sarg. in central New Mexico. The myceliophagous nematode, an undescribed species of *Aphelenchoides*, was isolated from a *J. monosperma* rootlet in August 1965. *Suillus granulatus* was isolated from sporophore tissue in July 1965; *Mycelium radicis atrovirens* was isolated from mycorrhizae of *P. ponderosa* in June 1965.

In the first experiment, nematodes were added axenically to the fungi immediately after the fungi were placed on potato-dextrose agar (PDA) in 90-mm petri dishes. Twenty-four 5-mm discs of each of the two fungi were cut from 3-week-old cultures with a No. 2 cork borer and placed on agar in the center of individual petri dishes. Twelve discs of each fungus were inoculated with similar discs obtained from a nematode-infected culture, and the remaining 12 discs of each fungus served as controls.

To determine whether the nematodes could maintain themselves on PDA without a fungus, 12 additional petri dishes were prepared. Six of these dishes were inoculated with nematodes obtained from a culture of *M. radicis atrovirens;* the remaining six were inoculated with nematodes from a *S. granulatus* culture.

In a second experiment, the addition of nematodes was delayed until the fungi had grown 4 days. The fungi were transferred and inoculated with nematodes by the same techniques used in the first experiment.

Diameter growth of the substrate and aerial mycelium of nematode-inoculated and nematode-free fungus cultures was measured in millimeters every 4 days. Substrate mycelial growth refers to growth of mycelium within the agar medium, whereas aerial mycelial growth refers to growth above the agar surface.

All dishes were completely randomized on a laboratory table, and each dish that contained nematodes was examined microscopically every 4 days for the presence of eggs. The experiments were continued for 40 days. Ambient air temperature ranged from 20 to 24 C and averaged 22 C.

After the final diameter-growth measurements were taken, a small piece of mycelium from each nematode-inoculated fungus culture was transferred to PDA to determine whether the fungi had been destroyed.

Nematodes were recovered from the fungus-nematode cultures by the Baermann funnel technique. Each culture was diced and placed in a funnel for 48 hr. The water in which the nematodes had collected was then drawn off and diluted to a known volume, and the nematodes in three to six 1-ml aliquots from each culture were counted.

RESULTS.—The nematode greatly reduced growth of the aerial and substrate mycelium of *M. radicis atrovirens*. When the nematodes were added immediately after this fungus was placed on agar, a reduction in diameter growth of substrate and aerial mycelium was apparent in 4 days (Fig. 1-A). In 40 days, the diameter of the substrate mycelium was only 38 mm (Fig. 1-A; 2), whereas the aerial mycelium was gradually destroyed until it measured only 6 mm. By contrast, the substrate and aerial mycelium of the nematode-free fungus had grown to the limits of the 90-mm petri dish in 32 days.

When the addition of nematodes was delayed until *M. radicis atrovirens* had grown 4 days, there was no

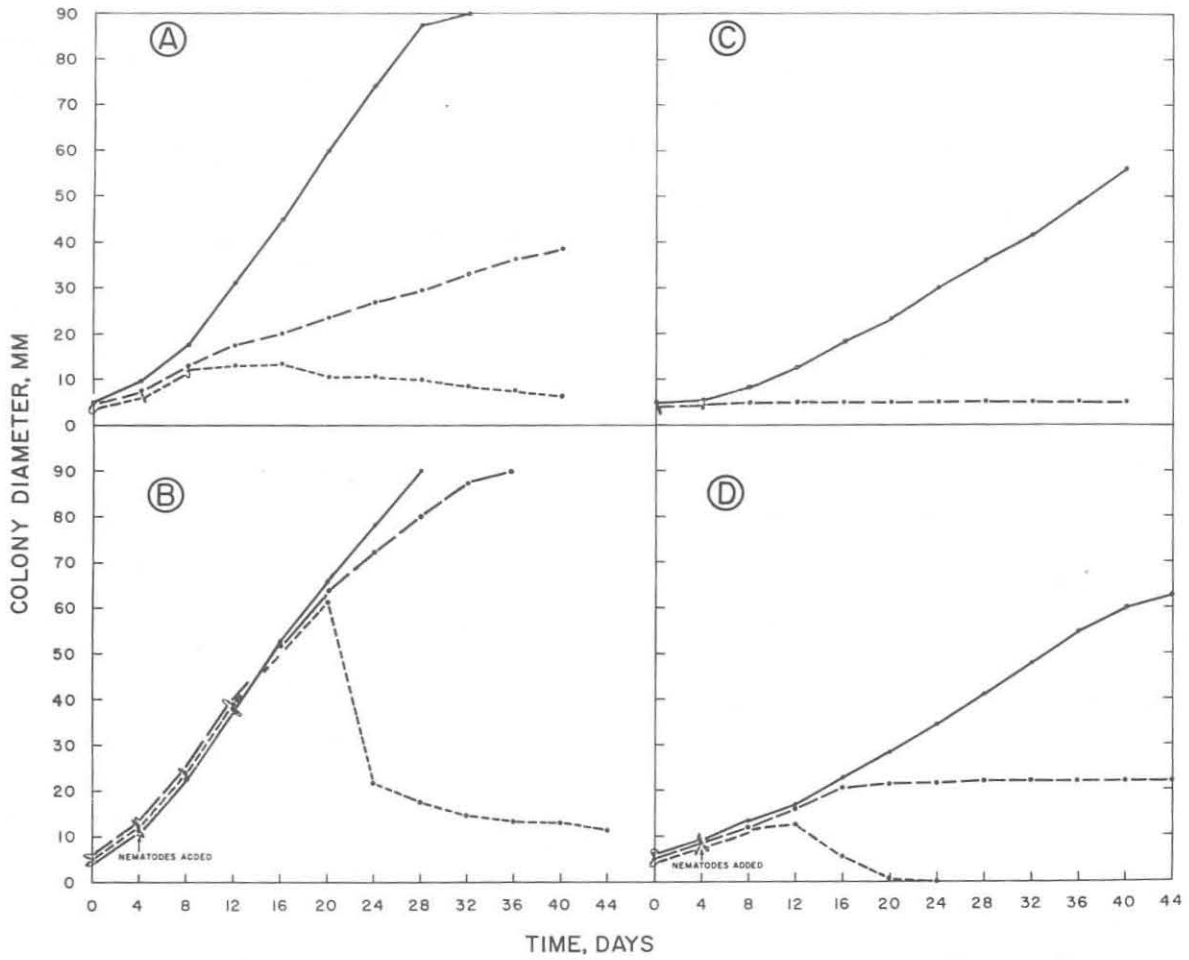

Fig. 1. Effect of myceliophagous nematodes on the growth of a pseudomycorrhizal and a mycorrhizal fungus. **A, B)** *Mycelium radicis atrovirens:* A) Nematodes added immediately after the fungus was placed on an agar medium, and B) nematodes added after the fungus had grown 4 days. **C, D)** *Suillus granulatus:* C) Nematodes added immediately after the fungus was placed on an agar medium, and D) nematodes added after the fungus had grown 4 days. The solid line represents substrate and aerial mycelial growth of the nematode-free fungus; the broken line represents substrate mycelial growth of the nematode-inoculated fungus; and the dashed line represents aerial mycelial growth of the nematode-inoculated fungus.

effect on the diameter growth of the substrate and aerial mycelium until 20 days (Fig. 1-B). After this time, the rate of diameter growth of the substrate mycelium slowed, but growth continued to the limits of the petri dish in 36 days. The diameter of the aerial mycelium of the nematode-inoculated fungus declined rapidly between the 20th and 24th days, and at the end of the experiment measured 11 mm. The substrate and aerial mycelial growth of the nematode-free fungus completely covered the petri dish in 28 days.

The diameter growth of the substrate and aerial mycelium of *S. granulatus* also was markedly reduced by nematode feeding. When nematodes were added immediately after this fungus was placed on PDA, the fungus did not grow (Fig. 1-C). However, when the addition of nematodes was delayed 4 days, diameter growth of the substrate mycelium was slowed markedly after 16 days (Fig. 1-D) and in 20 days reached a plateau of 22 mm and showed no further increases. The first reduction in the diameter of the aerial mycelium occurred after 12 days, and in 24 days all the aerial mycelium was destroyed (Fig. 1-D; 3).

The nematodes destroyed 87% of the *S. granulatus* cultures. Only 3 of 24 cultures grew when transferred to a fresh agar medium at the end of the experiments. These three cultures were from the experiment in which the addition of nematodes was delayed until the fungus had grown 4 days. All nematode-inoculated *M. radicis atrovirens* cultures grew when transferred to a fresh agar medium.

Nematode populations increased readily on both fungi (Table 1). Males were not observed in the cultures. Nematode eggs were observed on the agar surface of cultures of both fungi from 4 to 40 days after the nematodes were added.

Nematode populations in dishes that contained PDA without a fungus declined almost to zero during the experiments (Table 1). Nematode eggs were not ob-

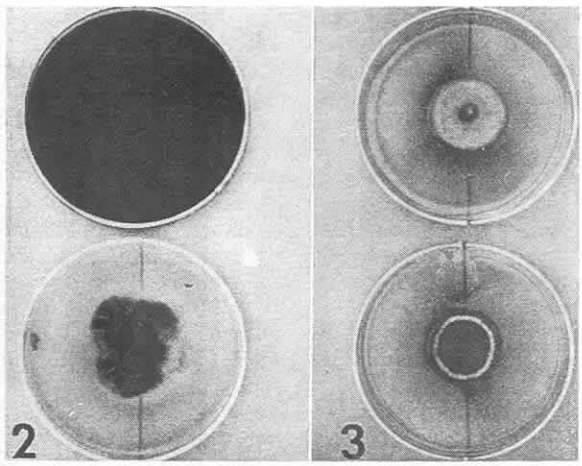

Fig. 2, 3. Effect of myceliophagous nematodes on the growth of a pseudomycorrhizal and a mycorrhizal fungus. **2)** Reduction in diameter growth of substrate mycelium of *Mycelium radicis atrovirens* due to nematode feeding (40 days). Upper: Nematode-free fungus covering entire petri dish. Lower: Nematode-inoculated fungus with a diameter of 38 mm. **3)** Destruction of aerial mycelium of *Suillus granulatus* due to nematode feeding (16 days). Upper: Nematode-free fungus. Lower: Nematode-inoculated fungus in which a small ring of aerial mycelium remains.

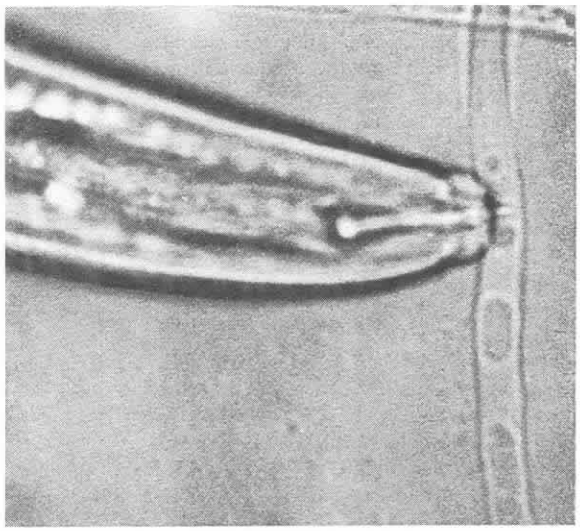

Fig. 4. An *Aphelenchoides* species feeding on a hypha of *Suillus granulatus*. Note bubbles occurring in the hypha at the feeding site.

served on the agar surface in these dishes. The nematodes remained active for 20 to 28 days, but then their activity gradually declined and they appeared moribund on the agar surface when the experiments were concluded.

Nematodes concentrated at the periphery of the fungus colonies and fed on surface as well as substrate hyphae. Upon locating a hyphal cell, the nematode penetrated the wall with its spear after about 5-10 thrusts and fed on the cellular contents for 5 to 60 sec. Bubbles usually appeared in the hyphal cell at the feeding site (Fig. 4), and the median bulb valve moved rapidly at the end of each feeding.

Discussion.—The effects of nematode feeding on the growth of the two fungi were similar in most respects. Diameter growth of substrate and aerial mycelium was reduced most when nematodes were added immediately after the fungi. When the nematodes were added after the fungi had grown 4 days, their effect on substrate and aerial mycelium was delayed. The observed delay in effect on the diameter growth of the fungi represents the time required by the nematodes to feed on and destroy the additional area of fungal growth. In feeding, the nematodes destroyed the fungal growth centrifugally, so that aerial mycelial growth declined before substrate mycelial growth (Fig. 3). An additional factor which probably contributed to the observed delay for *M. radicis atrovirens* was the difference in the initial nematode populations used in the two experiments, caused by differences in nematode populations in the petri dishes used for inoculum.

On *S. granulatus*, the nematode population increased only sixfold when the nematodes were added immediately after the fungus. This low population increase may be attributed to the number of nematodes initially added to the fungus culture. Apparently there were enough nematodes to feed on and destroy the 5-mm fungus disc before any growth occurred. Then, however, many of the nematodes gradually starved, and

Table 1. Nematode populations on *Mycelium radicis atrovirens* and *Suillus granulatus* in two laboratory experiments

Treatment	Nematode population		
	Initial[a]	Final[a]	Increase
Nematodes added immediately after fungus			
M. radicis atrovirens	2,215 ± 42 (20)	139,333 ± 6,353 (60)	63×
Control (nematodes only)	236 ± 10 (10)	<1 (6)	0
S. granulatus	943 ± 193 (12)	5,467 ± 785 (33)	6×
Control (nematodes only)	865 ± 28 (10)	0 (6)	0
Nematodes added 4 days after fungus			
M. radicis atrovirens	461 ± 30 (12)	461,027 ± 15,161 (72)	1,000×
Control (nematodes only)	68 ± 16 (10)	0 (6)	0
S. granulatus	1,022 ± 68 (12)	103,806 ± 6,187 (72)	102×
Control (nematodes only)	221 ± 8 (10)	0 (6)	0

[a] Numbers listed in columns 2 and 3 represent the mean population ± the standard error of the mean, with the number of observations in parentheses.

these were not recovered by the technique used at termination of the experiments. The above observation is substantiated by the fact that the nematodes were not able to maintain themselves in other cultures that contained an agar medium without the fungus.

When the addition of nematodes to *S. granulatus* was delayed until the fungus had grown for 4 days, 75% of the cultures were destroyed. Anderson (1) reported that cytoplasmic changes occurring during and after feeding of *Ditylenchus destructor* Thorne, 1945 resulted in the death of fungal cells of three small-celled fungi. Similar phenomena were observed in the present study.

Myceliophagous nematodes may have an indirect role in the premature mortality of low-elevation *Pinus ponderosa* and its woodland associates that occurs following periods of prolonged drought in New Mexico. Exploratory research indicates that plant-parasitic nematodes, root-staining and decay fungi, and a deficiency of mycorrhizae may be the most highly significant factors in predisposing trees to insect attacks and death. Mycorrhizae are important in the growth and survival of trees because they improve the absorption of nutrients from the soil, and probably protect the root against pathogenic fungi. There is also some evidence that mycorrhizae may increase the drought tolerance of pines (3). The nematodes possibly damage or destroy mycorrhizae, which may result in reduced tree vigor and limited root extension in the soil.

The present investigation reveals that a myceliophagous nematode markedly reduces the growth of a mycorrhizal fungus under laboratory conditions. Further laboratory and greenhouse studies are necessary, however, to evaluate the effects of these nematodes on the incidence and development of mycorrhizae of southwestern tree species.

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